EXHIBIT JJ

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United States Patent [19]

Mitsuhashi et al.

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[45] Date of Patent: Jun. 17, 1997

[54] **METHOD FOR DETECTING POLYNUCLEOTIDES WITH IMMOBILIZED POLYNUCLEOTIDE PROBES IDENTIFIED BASED ON T_M**

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[21] Appl. No.: **379,078**

[22] Filed: **Jan. 26, 1995**

Related U.S. Application Data

[63] Continuation of Ser. No. 974,406, Nov. 12, 1992, abandoned, which is a continuation-in-part of Ser. No. 922,522, Jul. 28, 1992, abandoned.

[51] **Int. Cl.**[6] **C12Q 1/68**; C07H 21/04

[52] **U.S. Cl.** **435/6**; 536/24.3; 536/24.31; 536/24.32

[58] **Field of Search** 435/6, 5, 810; 536/24.3, 24.31, 24.32; 935/78

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[57] **ABSTRACT**

A method for detecting the presence of a particular organism, infectious agent, or biological component of a cell or organism in a sample, based on sandwich hybridization in which first and second probes are used, and the specificity of the first probe is determined based on its melting temperature (T_m) with the target polynucleotide at a selected sodium and formamide concentration.

37 Claims, 63 Drawing Sheets

component of a cell or organism in a biological sample comprises the steps of:

(a) immobilizing a first polynucleotide probe to a solid support, wherein the nucleotide sequence of the first polynucleotide probe is sufficiently complementary to a first nucleotide sequence contained in an analyte polynucleotide in the organism, infectious agent, or biological component that the first polynucleotide probe can hybridize to the first nucleotide sequence of the analyte polynucleotide of the organism, infectious agent, or biological component;

(b) contacting the polynucleotides present in the sample with the first polynucleotide probe;

(c) hybridizing the analyte polynucleotide in the sample to the first polynucleotide probe, if the analyte polynucleotide is present in the sample;

(d) contacting a second polynucleotide probe with the analyte polynucleotide hybridized to the first polynucleotide probe, if the analyte polynucleotide from the sample has hybridized to the first polynucleotide probe, wherein the nucleotide sequence of the second polynucleotide probe is sufficiently complementary to a second nucleotide sequence contained in the analyte polynucleotide of the organism, infectious agent, or biological component that the second polynucleotide probe can hybridize to the second nucleotide sequence;

(e) hybridizing the second polynucleotide probe to the analyte polynucleotide hybridized to the first polynucleotide probe, if the analyte polynucleotide has hybridized to the first polynucleotide probe; and

(f) determining the presence of the organism, infectious agent, or biological component in the sample by detecting the presence of the second polynucleotide probe hybridized to the analyte polynucleotide which has hybridized to the first polynucleotide probe.

In this method, the second polynucleotide probe can preferably have the same or lower T_m as the first polynucleotide probe. Preferably, the first polynucleotide probe also has a T_m within the range of from approximately 48° C. to approximately 60° C. The first nucleotide sequence of the analyte polynucleotide can also in one embodiment be common to a plurality of organisms, infectious agents, or biological components of a cell or organism. Alternatively, the first nucleotide sequence of the analyte polynucleotide can be specific to a particular organism, infectious agent, or biological component of a cell or organism.

The second nucleotide sequence of the analyte polynucleotide can have a sequence common to a plurality of organisms, infectious agents, or biological components of a cell or organism in this embodiment of the present method. In an alternative embodiment, the second nucleotide sequence of the analyte polynucleotide can have a sequence that is specific for a particular organism, infectious agent, or biological component of a cell or organism. Additionally, a label can advantageously be attached to the second polynucleotide probe. Any of a number of polynucleotide labels known to the art can be used, including a radionuclide, an enzyme, an enzyme substrate, a specific binding moiety, an binding partner for a specific binding moiety, biotin, avidin, a nucleic acid stain, or a fluorescent material. If a nucleic acid stain is used as the label, the stain can consist of either ethidium bromide, yoyo-1, or toto-1. When the label is a light-emitting substance, the label can advantageously be detected by measuring the amount of light emitted therefrom. When measuring the amount of light emitted by the label, light can be recorded on film, after which the amount of exposure of the film is measured using a densitometer. In an even more preferable embodiment, the label comprises alkaline phosphatase and the label is detected by adding ATTOPHOS to the solution containing the labeled probe and then measuring the fluorescence emitted using a fluorimeter.

A solid support such as a microtiter plate having a plurality of wells can be used to perform the present method. Preferably, each of the wells has a specific polynucleotide probe immobilized thereon. The first polynucleotide probe, which can be immobilized on the microtiter plate, advantageously comprises DNA, and more advantageously both the first and second polynucleotide probes comprise DNA.

A further preferable step of the method of the present invention involves washing the solid support after hybridizing the analyte polynucleotide in the sample to the first polynucleotide probe. In this way, substantially all of the biological sample not annealed to the first polynucleotide probe is removed from the solid support. Yet another step of the method includes the step of washing the solid support after hybridizing the second polynucleotide probe with the analyte polynucleotide, which is itself hybridized to the first polynucleotide probe. After such washing, substantially all of the second polynucleotide probe not hybridized with the analyte polynucleotide is removed from the solid support. The analyte polynucleotide in this method can be selected from the group consisting of mRNA, rRNA, and genomic DNA.

Another embodiment of the present invention is a method of detecting the presence of an organism, infectious agent, or biological component of a cell or organism in a biological sample containing polynucleotides, comprising the steps of:

(a) identifying a first polynucleotide probe and a second polynucleotide probe, wherein the nucleotide sequence of the first polynucleotide probe is sufficiently complementary to a first nucleotide sequence contained in an analyte polynucleotide of the organism, infectious agent, or biological component that the first polynucleotide probe can hybridize to the first nucleotide sequence of the analyte polynucleotide of the organism, infectious agent, or biological component, and wherein the nucleotide sequence of the second polynucleotide probe is sufficiently complementary to a second nucleotide sequence contained in the analyte polynucleotide of the organism, infectious agent, or biological component that the second polynucleotide probe can hybridize to the second nucleotide sequence, the second nucleotide sequence being common to a plurality of organisms, infectious agents, or biological components;

(b) immobilizing the first polynucleotide probe to a solid support;

(c) contacting the polynucleotides present in the sample with the first polynucleotide probe;

(d) hybridizing an analyte polynucleotide in the sample to the first polynucleotide probe, if the analyte polynucleotide is present in the sample;

(e) contacting the second polynucleotide probe with the analyte polynucleotide hybridized to the first polynucleotide probe, if the analyte polynucleotide from the sample has hybridized to the first polynucleotide probe;

(f) hybridizing the second polynucleotide probe to the analyte polynucleotide hybridized to the first polynucleotide probe, if the analyte polynucleotide has hybridized to the first polynucleotide probe; and

(g) determining the presence of the organism, infectious agent, or biological component in the sample by detect-

enzyme substrate, a specific binding moiety, an binding partner for a specific binding moiety, biotin, avidin, a nucleic acid stain, and a fluorescent material. Additionally, the solid support-polynucleotide structure can even more advantageously have a second polynucleotide probe that is common to polynucleotides contained in a plurality of organisms, infectious agents, or biological components of a cell or organism. In still another preferred embodiment of this method, the first and second polynucleotide probes are determined through the use of a computer system for designing oligonucleotide probes for use with a gene sequence data source. This the computer system can comprise an input means for retrieving the gene sequence data, a processor, and instructions directing the processor to determine the first oligonucleotide probe. Yet still more preferably, the solid support-polynucleotide structure of the above method has a polynucleotide selected from the group consisting of mRNA, rRNA, and genomic DNA.

Another embodiment of the present invention is a kit for identifying the presence of an organism, infectious agent, or biological component of a cell or organism in a biological sample which contains:

a specific polynucleotide probe, the specific polynucleotide probe being complementary to or homologous to a first nucleotide sequence in an analyte polynucleotide specific to a particular organism, infectious agent, or biological component to be detected; and

a common polynucleotide probe complementary to or homologous to a second nucleotide sequence in the analyte polynucleotide of the organism, infectious agent, or biological component, the common polynucleotide probe being complementary to polynucleotides contained in a plurality of organisms, infectious agents, or biological components.

In addition, the above kit can advantageously have a solid support to which a polynucleotide can be immobilized. Even more preferably, the kit has a specific polynucleotide probe immobilized to the solid support. Still more advantageously, the above kit has a solid support with a plurality of specific polynucleotide probes immobilized thereto, each of the probes specific to a different organism, infectious agent, or biological component. Most preferably, the solid support of the kit has a plurality of wells, each of the specific polynucleotide probes being immobilized to a different well, and a buffer appropriate for the hybridization of the probes and polynucleotides, with the polynucleotides being selected from the group consisting of mRNA, rRNA, and genomic DNA.

Similarly, the second polynucleotide probe of the above kit can advantageously bear a label, with the label being selected from the group consisting of a radionuclide, an enzyme, an enzyme substrate, a specific binding moiety, an binding partner for a specific binding moiety, biotin, avidin, a nucleic acid stain, and a fluorescent material. Further, the above kit can have a specific polynucleotide probe comprising a first specific primer which is complementary or homologous to a sequence specific to a particular organism, infectious agent, or biological component, the kit additionally comprising a second specific polynucleotide primer which is complementary or homologous to a different sequence specific to the organism, infectious agent, or biological component.

When the above kit is designed to be used with PCR, it should have one or more of the following: dNTP's, a reverse transcriptase, a polymerase, and a buffer appropriate for the addition of dNTP's to a primer using a reverse transcriptase or polymerase. Additionally, the kit can include a DNA polymerase that has significant polymerase activity at temperatures above 50° C.

In yet another embodiment, the present method comprises a means of detecting the presence of one or more organisms, infectious agents, or biological components in a biological sample containing polynucleotides, wherein at least one of the polynucleotides is indicative of the presence of the one or more organisms, infectious agents or biological components and is present in minute quantities. This method makes use of PCR and employs the steps of:

(a) obtaining a biological sample containing polynucleotides;

(b) contacting the sample with a first polynucleotide primer, the first primer having a nucleotide sequence complementary to a nucleotide sequence common to a plurality of organisms, infectious agents, or biological components;

(c) hybridizing the first primer to an analyte polynucleotide present in the sample that is complementary to the first primer, if such an analyte polynucleotide is present;

(d) extending the first primer, thereby producing a double-stranded polynucleotide including a complementary nucleotide strand comprising the first primer and having a nucleotide sequence complementary to the analyte polynucleotide;

(e) contacting the sample with a second polynucleotide primer, the second primer being complementary to a sequence contained in the complementary nucleotide strand;

(f) hybridizing the second primer to the complementary nucleotide strand;

(g) extending the second primer to form a nucleotide strand homologous to the analyte polynucleotide;

(h) contacting the sample with a third and a fourth polynucleotide primer, the third and fourth primers having sequences complementary to the homologous nucleotide strand and the complementary nucleotide strand, respectively, wherein the third primer has a nucleotide sequence complementary to a sequence common to a plurality of organisms, infectious agents, or biological components whose presence is to be determined and wherein the sequence of the third primer is different from that of the first primer;

(i) hybridizing the third and fourth primers to the complementary nucleotide strand and the homologous nucleotide strand;

(j) extending the third and fourth primers, thereby producing double-stranded polynucleotides; and

(k) determining the presence of the one or more organisms, infectious agents, or biological components in the sample by detecting the extension of at least one of the first, second, third, or fourth primers.

In this method, the second primer can have a nucleotide sequence that is common to a plurality of the organisms, infectious agents, or biological components whose presence is being determined. This method is preferably practiced such that the extending and hybridizing steps are repeated a plurality of times. In this method, the extension step can be accomplished with a reverse transcriptase when the primer is bound to RNA, while this step is accomplished with a DNA polymerase when the bound polynucleotide is DNA. If a DNA polymerase is used, it preferably has significant polymerase activity at temperatures above 50° C. In the present method the nucleotide sequences of the first and

in any way known to the art. Preferably, the organism suspected of carrying an infectious agent or other organism is a human, and the identification is made by a physician who observes symptoms indicative of the presence of such an organism or agent in such a human. For example, a patient diagnosed as having AIDS who comes down with pneumonia and who does not respond to anti-bacterial agents is identified by a physician as possibly harboring the fungus *Pneumocystis carinii*.

Alternatively, a biological sample taken from a host with no overt signs of having a medical condition or harboring an organism or agent can be tested. For example, a food sample or tissue from an AIDS patient without signs of a fungal growth can be tested for the presence of a fungus. In this embodiment, any biological sample can be tested, even though overt signs of the presence of a fungus are lacking in that sample. Appropriate action may thereby be taken if a fungus is in fact found in such a biological sample.

The biological sample to be tested can be obtained by any means known to the art. For example, if an AIDS patient is suspected of suffering from interstitial plasma pneumonia caused by the fungus *Pneumocystis carinii*, a sputum sample can be taken from the lungs of that patient. The sputum can be obtained by having the patient cough up phlegm from the lungs and deposit it into a cup. Alternatively, a sputum sample can be obtained by scraping the bronchial passage with a sterile swab, or by any other means known to the art. Any other biological sample which could possibly carry a fungus is likewise obtained in an appropriate fashion.

IV. Preparing the Biological Sample

The biological sample is next prepared so that the RNA and/or DNA present in the biological sample can be probed. When a fungus is being probed for, ribosomal RNA of any fungi present in the sample can be probed in accordance with the methods of the present invention. In order to probe the ribosomal RNA of any fungal cells present, these cells should first be lysed. Lysis of fungal cells or of other cells containing RNA or DNA of interest can be accomplished by any of a number of methods known to the art, including those set out in Molecular Cloning.

In one embodiment, the cells are lysed before they come into contact with the solid support. This embodiment might be used, for example, when the solid support is one which is not designed to hold a sample of lysed cells, such as a nitrocellulose filter. In this embodiment, the cells are contacted with the solid support after they have been lysed.

A variety of techniques can be used for cell lysis. When the ribosomal RNA of fungi is being probed, for example, techniques that separate the ribsomal RNA from the ribosomal proteins are preferred. Example 1 is provided to show one technique believed to be useful in obtaining ribosomal RNA. However, techniques for obtaining ribosomal RNA are well known. Techniques for obtaining DNA and other kinds of RNA are also well known to those of skill in the art. Thus, the technique of Example 1 is not necessarily a preferred method of obtaining ribosomal RNA-containing samples. Example 1, like all of the examples provided herein, are provided merely to illustrate certain aspects of the present invention. As such, they are not intended to limit the invention in any way.

EXAMPLE 1

Lysing Cells in a Biological Sample

Cells present in a biological sample can be lysed by treatment with a solution of 10 mM ethylenediaminetetraacetic acid (EDTA) (pH 8.0), 0.2M NaCl, 0.5% of sodium dodecyl sulfate (SDS), 500 Unit/ml of RNase inhibitor, 10 mM of Vanadyl Ribonucleosyl Complex and 200 μg/l of Proteinase K (hereafter called Lysis Buffer). After lysis of the cells, the NaCl concentration of the resulting cell lysate in solution is adjusted to 0.5M.

V. Solid Support

In a preferred embodiment, the solid support is capable of containing a biological sample and is resistant to the reagents used to lyse cells in the biological sample. The sample can thus be lysed in the solid support. However, the use of such a solid support is not necessary if the sample can be obtained without lysis or is lysed in a separate container. An example of a solid support that is resistant to a large number of treatments is a microtiter well or a plate made from a resistant plastic material.

The solid support can also be any of a variety of other solid supports known to the art, such as a membrane filter, a bead, or any other solid, insoluble support to which polynucleotides can be attached. The solid support is preferably made of a material which can immobilize a polynucleotide probe. Immobilization can be through covalent bonds or through any of a variety of interactions that are known to those having skill in the art. Plastic materials containing carboxyl or amino groups on their surfaces, such as polystyrene, are preferred for the solid support of the present invention because polynucleotide probes can be immobilized on their surfaces, because they are inexpensive and easy to make, and because they are resistant to the reagents used to lyse the cells of the biological samples used in the present invention. For example, the Sumilon microtiter plate MS-3796F made by Sumitomo Bakelite, which has a carboxyl group on its surface, can be used in such a preferred embodiment. A plastic plate having an amino group on its surface, such as the Sumilon microtiter plate MS-3696, can also be used.

VI. Contacting the Sample and First Polynucleotide Probe

After the cells in the biological sample have been lysed, the RNA and/or DNA contained in such cells is substantially released into solution or otherwise made available to being probed. If the biological sample was not lysed in the solid support, the cell lysate is next brought into contact with the solid support. Immobilized to the solid support is a first polynucleotide probe which preferably contains a sequence complementary to a specific sequence in the RNA and/or DNA of a particular organism, infectious agent, or biological component of a cell or organism in the biological sample. In an alternate embodiment, the first polynucleotide probe can also contain a sequence common to a plurality of organisms, infectious agents, or biological components when any of a group of such organisms, infectious agents, or biological components is sought to be identified. When the RNA and/or DNA present in the cell lysate contacts the solid support, therefore, it also comes into contact with the first polynucleotide probe.

Preferably, the first polynucleotide probe is an oligodeoxyribonucleotide (DNA) rather than an oligoribonucleotide (RNA), since DNA is more stable than RNA. The number of nucleotides in the polynucleotide probe is not restricted. However, if an oligodeoxynucleotide is used as the specific polynucleotide probe, a preferred length for the oligodeoxynucleotide is from 15 to 100 nucleotides. Lengths longer than 100 nucleotides are usable within the scope of the present invention. However, lengths of 100 nucleotides or less are preferable because many automated polynucleotide synthesizers have a limit of 100 nucleotides. Longer sequences can be obtained by ligating two sequences of less than 100 nucleotides.

EXHIBIT LL

D28a

OPPOSITION BY AFFYMETRIX, INC
AGAINST EUROPEAN PATENT NO 0 373 203
OF ISIS INNOVATION LIMITED
NOTICE AND STATEMENT

A. General

Affymetrix, Inc, a Californian company, of 3380 Central Expressway, Santa Clara, California 95051, USA (hereinafter "the Opponent") hereby opposes European Patent No 0 373 203 (hereinafter "the Patent") of Isis Innovation Limited (hereinafter "the Patentee") to the extent of each and every claim.

The Opponent requests that in the event the Opposition Division should have in mind maintenance of the Patent, Oral Proceedings should be appointed. Obviously, the Opponent fully reserves its position on costs if this should prove to be appropriate. The Opponent also fully reserves its position regarding further or other submissions should the Patentee offer amendments.

B. Grounds

The Grounds of Opposition are, broadly stated:

(a) the subject-matter of the Patent is not patentable within the terms of Articles 52 to 57 EPC;

the β-globin gene are shown at pp. 19-20. Thus, claim 3 is anticipated by D2.

Claim 4 adds the limitation to claim 3 that the one or more pairs of oligonucleotides represent "normal and mutant versions of a point mutation". Because the sickle-cell mutation is a point mutation, this claim lacks novelty as with claim 3.

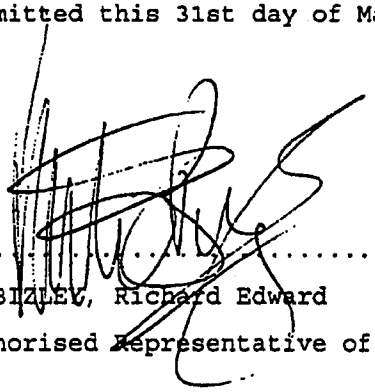
Claim 7 adds to claims 1 to 6 the additional limitation that the chosen length of the oligonucleotides is from 8-20 nucleotides. As already noted in connection with claim 1, D2 provides an example where an oligonucleotide probe has a length of 16 nucleotides. Claim 7 thus lacks novelty over D2.

Claim 8 adds to claims 1 to 7 the additional limitation that "each oligonucleotide is bound to the support by a covalent link through a terminal nucleotide". D2 discloses that it "is well known that noncovalent immobilization of an oligonucleotide is ineffective on a solid support." (P. 9, line 34). Further, D2 describes methods for achieving covalent modification via a terminal nucleotide to a support, the support optionally including a linker (pp. 18-19). Accordingly, claim 8 lacks novelty over D2.

Claim 9 adds to claims 1-8 the further limitation that the "oligonucleotide in each cell has a defined sequence." The term "defined" indicates that some property of the sequence is known but does not

desired array has been generated", and , as demonstrated above, such would not in many/most cases have been possible.

Submitted this 31st day of May 1995



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by BIZLEY, Richard Edward

Authorised Representative of the Opponent

EXHIBIT MM

08/327525

Attorney Docket No. 16528X-82/1091

PATENT APPLICATION

COMPUTER-AIDED VISUALIZATION AND ANALYSIS SYSTEM FOR SEQUENCE EVALUATION

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WHAT IS CLAIMED IS:

CallSeq™

1. In a computer system, a method of identifying an unknown base in a sample nucleic acid sequence, said method comprising the steps of:

inputting a plurality of probe intensities, each of said probe intensities being associated with a probe on a chip;

said computer system comparing said plurality of probe intensities wherein each of said plurality of probe intensities is substantially proportional to a probe hybridizing with at least one sequence; and

calling said unknown base according to said comparison of said plurality of probe intensities.

2. The method of claim 1, wherein said at least one sequence includes said sample sequence.

3. The method of claim 2, further comprising the step of said computer system calculating a ratio of a higher probe intensity to a lower probe intensity.

4. The method of claim 3, further comprising the step of calling said unknown base as being a base complement of said probe associated with said higher probe intensity if said ratio is greater than a predetermined ratio value.

5. The method of claim 3, wherein said ratio is approximately 1.2.

6. The method of claim 2, further comprising the step of sorting said plurality of probe intensities.

7. The method of claim 1, wherein said at least one sequence includes said sample sequence and a reference sequence.

8. The method of claim 7, further comprising the step of said computer system comparing probe intensities of a probe hybridizing with said sample sequence to probe intensities hybridizing with said reference sequence.

9. The method of claim 7, further comprising the step of calculating first ratios of a wild-type probe intensity to each probe intensity of a probe hybridizing with said reference sequence, wherein said wild-type probe intensity is associated with a wild-type probe.

10. The method of claim 9, further comprising the step of calculating second ratios of the highest probe intensity of a probe hybridizing with said sample sequence to each probe intensity of a probe hybridizing with said sample sequence.

11. The method of claim 10, further comprising the step of calculating third ratios of said first ratios to said second ratios.

12. The method of claim 7, further comprising the step of comparing neighboring probe intensities of said plurality of probe intensities.

13. The method of claim 7, wherein probe intensities of a probe hybridizing with said reference sequence are from a plurality of experiments.

14. The method of claim 13, further comprising the step of said computer system comparing probe intensities of a probe hybridizing with said sample sequence to statistics about said plurality of experiments.

15. The method of claim 14, wherein said statistics include a mean and standard deviation.

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16. The method of claim 13, further comprising the step of normalizing said plurality of probe intensities by dividing each probe intensity by a sum of related probe intensities.

17. The method of claim 1, further comprising the step of subtracting a background intensity from each of said plurality of probe intensities.

18. The method of claim 1, further comprising the step of setting a probe intensity equal to a relative small positive number if said probe intensity is less than or equal to zero.

19. The method of claim 1, further comprising the step of indicating said unknown base is unable to be called if said plurality of probe intensities have insufficient intensity to call said unknown base.

20. The method of claim 1, wherein said unknown base is called as being A, C, G, or T.

Pooling Processing

21. A method of processing first and second nucleic acid sequences, comprising the steps of:

providing a plurality of nucleic acid probes;

labeling said first nucleic acid sequence with a first marker;

labeling said second nucleic acid sequence with a second marker; and

hybridizing said first and second labeled nucleic acid sequences at the same time.

22. The method of claim 21, wherein said plurality of nucleic acid probes are on a chip.

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23. The method of claim 21, further comprising the step of fragmenting said first and second nucleic acid sequences at the same time.

24. The method of claim 21, further comprising the step of scanning for said first and second markers on said chip, said first and second labeled nucleic acid sequences being on said chip.

25. The method of claim 21, wherein said first and second markers are fluorescent markers.

26. The method of claim 25, wherein said first and second markers emit light at different wavelengths upon excitation.

ViewSeq™

27. In a computer system, a method of analyzing a plurality of sequences of bases, said plurality of sequences including at least one reference sequence and at least one sample sequence, the method comprising the steps of:

displaying said at least one reference sequence in a first area on a display device; and

displaying said at least one sample sequence in a second area on said display device;

whereby a user is capable of visually comparing said plurality of sequences.

28. The method of claim 27, wherein said plurality of sequences are monomer strands of DNA or RNA.

29. The method of claim 27, wherein said bases are A, C, G, or T.

30. The method of claim 27, wherein said at least one reference sequence includes a chip wild-type that has been tiled on a chip.

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31. The method of claim 30, wherein said chip wild-type sequence is displayed as a first sequence in said first area.

32. The method of claim 30, further comprising the step of displaying a label in said first area to identify said chip wild-type sequence.

33. The method of claim 32, wherein said label is a capital C.

34. The method of claim 27, wherein said at least one sample sequence has been hybridized on a chip.

35. The method of claim 27, further comprising the step of indicating bases that differ among a plurality of user selected sequences.

36. The method of claim 27, further comprising the steps of:

displaying a name associated with each of said at least one reference sequence in said first area; and

displaying a name associated with each of said at least one sample sequence in said second area.

37. The method of claim 27, further comprising the step of linking at least one reference sequence in said first area with at least one sample sequence in said second area.

38. The method of claim 37, further comprising the step of indicating on said display device which sequences are linked.

39. The method of claim 38, wherein said indicating step includes the step of displaying a common symbol next to said linked sequences.

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40. The method of claim 39, wherein said common symbol is a link number.

41. The method of claim 37, further comprising the step of indicating bases of said at least one sample sequence that are not equal to a corresponding base in said at least one reference sequence.

42. The method of claim 27, wherein said at least one reference sequence and said at least one sample sequence are aligned on said display device.

43. The method of claim 27, further comprising the step of exposing sequences to probes.

44. The method of claim 43, further comprising the step of evaluating said exposed sequences according to hybridization with said probes.

add B1

add C1

EXHIBIT NN

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/327,525	10/21/94	CHEE	M 16528X82

EXAMINER: REES, D

ART UNIT	PAPER NUMBER
1807	7

DATE MAILED: 09/19/95

18N2/0919

VERN NORVIEL
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This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☐ This application has been examined ☐ Responsive to communication filed on _____ ☐ This action is made final.

Restriction

A shortened statutory period for response to this action is set to expire 1 month(s), 0 days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☐ Notice of References Cited by Examiner, PTO-892.
2. ☐ Notice of Draftsman's Patent Drawing Review, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____

Part II SUMMARY OF ACTION

1. ☒ Claims 1-44 are pending in the application.
   Of the above, claims _____ are withdrawn from consideration.
2. ☐ Claims _____ have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☐ Claims _____ are rejected.
5. ☐ Claims _____ are objected to.
6. ☒ Claims 1-44 are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice of Draftsman's Patent Drawing Review, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____, has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed _____, has been ☐ approved; ☐ disapproved (see explanation).
12. ☐ Acknowledgement is made of the claim for priority under 35 U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____.
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

EXAMINER'S ACTION

PTOL-326 (Rev. 2/93)

Part III DETAILED ACTION

Election/Restriction

1. Restriction to one of the following inventions is required under 35 U.S.C. 121:

Group I. Claims 1-20, drawn to a method of identifying an unknown base (CallSeq™), classified in Class 364, subclass 496.

Group II. Claims 21-26, drawn to a method of processing first and second nucleic acid sequences ("Pooling Processing"), classified in Class 435, subclass 6, for example.

Group III. Claims 27-44, drawn to a method of analyzing a plurality of sequences (ViewSeq™) , classified in Class 435, subclass 91.1.

2. The inventions are distinct, each from the other because of the following reasons:

Inventions I, II and III are related as subcombinations disclosed as usable together in a single combination. The subcombinations are distinct from each other if they are shown to be separately usable. In the instant case, the inventions have

separate utilities in that the method of Invention I may be used to determine the sequence of a nucleotide, the method of invention II may be used in hybridization assays that do not involve sequencing and the method of Invention III may be used for gene mapping assays and genotyping. The methods further involve distinct method steps and provide different outcomes. See M.P.E.P. § 806.05(d).(i)

Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by their different classification, restriction for examination purposes as indicated is proper.

3. A telephone call was made to Michael Ritter on Sept 14, 1995 to request an oral election to the above restriction requirement, but did not result in an election being made.

4. Applicant is advised that the response to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed.

5. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 C.F.R. § 1.48(b) if one or more of the

currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filed petition under 37 C.F.R. § 1.48(b) and by the fee required under 37 C.F.R. § 1.17(h).

6. Papers related to this application may be submitted to Group 1800 by facsimile transmission via the P.T.O. Fax Center located in Crystal Mall 1. The CM1 Fax Center number is (703) 308-7939. Please note that the faxing of such papers must conform with the notice to Comply published in the Official Gazette, 1096 OG 30 (Nov 15, 1989).

An inquiry regarding this communication should be directed to examiner Dianne Rees, Ph.D., whose telephone number is (703) 308-6565. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, W. Gary Jones, can be reached on (703) 308-1156.

Calls of a general nature may be directed to the Group receptionist who may be reached at (703) 308-0196.

Dianne Rees

Dianne Rees

Sept 15, 1995

STEPHANIE W. ZITOMER
PRIMARY EXAMINER
GROUP 1800

EXHIBIT OO

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231,
on Oct. 19 1995
By Anne Currier Carr
Anne Currier Carr

PATENT

Attorney Docket No. 16528X-82

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

MARKS S. CHEE et al.

Serial No.: 08/327,525

Filed: October 21, 1994

For: COMPUTER-AIDED VISUALIZATION AND ANALYSIS SYSTEM FOR SEQUENCE EVALUATION

Examiner: [redacted]

Art Unit: [redacted]

RESPONSE TO RESTRICTION REQUIREMENT

RECEIVED
NOV 17 1995
GROUP 1800

RECEIVED
96 NOV -9 PM 4:51
GROUP 260

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to the Restriction Requirement mailed September 19, 1995, Applicants elect to pursue claims in Group I.

Also enclosed herewith is a Preliminary Amendment in which Applicants canceled the claims in the other Groups. New claims 45-59 are also added by the Preliminary Amendment; however, these new claims are clearly directed to subject matter similar to the claims in Group I.

Respectfully submitted,

Michael J. Ritter

Michael J. Ritter
Reg. No. 36,653

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EXHIBIT PP

EXHIBIT REDACTED IN ITS ENTIRETY